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THE EGGS OF *AEDES VIGILAX* AND *AEDES VITTIGER* (DIPTERA: CULICIDAE)

J. R. Linley, M. J. Geary, and R. C. Russell

(JRL) Florida Medical Entomology Laboratory, Institute of Food and Agricultural Sciences, University of Florida, 200 9th St. S.E., Vero Beach, Florida 32962; (MJG & RCR) Medical Entomology Unit, University of Sydney at Westmead Hospital, Westmead, N.S.W. 2145, Australia.

Abstract.—Descriptions based on scanning electron micrographs are given for the eggs of *Aedes* (*Ochlerotatus*) *vigilax* and *Ae.* (*Och.*) *vittiger.* There is pronounced lateral asymmetry in the egg of both species, with the dorsal surface flatter and the ventral surface more arched, especially in *Ae. vittiger.* In both cases the outer chorionic cells differ in size on the ventral, lateral and dorsal surfaces, but in *Ae. vittiger,* which exhibits a remarkable and distinctive overall surface uniformity, the structure of the tubercles within the cells is extremely constant. There are minor structural differences between cells on the different surfaces of *Ae. vigilax* eggs.

Key Words: Insecta, mosquito, eggs, fine structure, chorionic sculpturing

Aedes (*Ochlerotatus*) *vigilax* (Skuse) is an important coastal mosquito associated with low-lying estuarine land and mangrove swamps in the Australasian and Oriental regions. Typically, it breeds in very temporary bodies of brackish water formed by exceptionally high tides or rainfall accumulations in saline habitats. Although the larvae usually are found in water with some salt content, they have been found occasionally breeding in fresh water (Dobrotworsky 1965). The adults are vicious biters of man. This, as well as importance as a vector of disease organisms (see Lee et al. 1984), has made *Ae. vigilax* the subject of considerable research. Various studies of the egg stage have been made and the ecology of the egg has been investigated with respect to the natural distribution of eggs in the field as affected by soil wetness and the presence or absence of plants and shade (Reynolds 1961, Sinclair 1976, Kay and Jorgensen 1986). Pillai (1962) experimented with factors that affect survival of the egg and provided the first information on the egg's morphology by means of celloidin impressions of the chorion. Kay and Jorgensen (1986) partially described the fine structure of the egg with the aid of three electron micrographs, but did not give an account of variations in structure on the different surfaces as well as other details. We provide here a more complete description, enhanced by considerably more illustration.

Like *Ae. vigilax, Ae. vittiger* is a floodwater species, but in fresh rather than saline accumulations (Lee et al. 1984), where it prefers sunlight and bordering emergent grass (Marks 1967). The females actively attack man both by day and night and will feed also on a variety of animals (Lee et al. 1984). Multiplication of Murray Valley encephalitis virus takes place in females fed virus suspension (McLean 1953), and the species is considered a possible temporary or local vector of myxomatosis (Fenner and

Ratcliffe 1965). The biology of *Ae. vittiger* is less well known than *Ae. vigilax* and, apart from a celloidin impression of the mid-ventral chorion (Pillai 1962), the egg has not been described.

Materials and Methods

Eggs of both species were obtained from blood-fed females collected in New South Wales, Australia. Oviposition on filter paper was induced in the laboratory and several papers supporting eggs from a number of females were folded (while very damp) inside small petri dishes and mailed to Vero Beach. Groups of eggs for microscopy were prepared either by cutting out small pieces of paper bearing numbers of eggs and sticking these to stubs with silver paint, or by transferring single eggs with a fine artist's brush to stubs covered with double-sided sticky tape. Eggs from individual females could not be identified, but to increase the probability that eggs from several females were represented, specimens from widely separated areas of each egg paper were selected. Eight stubs were prepared for each species.

Once attached to stubs, eggs were dried over calcium chloride (0.5 h), coated with gold and examined in a Hitachi S-510 scanning electron microscope.

Where means (±SE) of dimensions and structures are given in the text, they were derived from 5 separate eggs selected so as to optimize the probability of each being from a separate female. The measurements were made from micrographs using a digitizing tablet and SigmaScan software (Jandel Scientific, Corte Madera, California). Cell dimensions were taken to the middle of the outer chorionic reticulum, lengths between the two points of the cell most widely separated approximately in the egg's longitudinal axis, widths between similar points circumferentially. Cell areas were obtained by digitizing the perimeter in each case. Tubercles were measured across the widest point. Analysis of variance and the Student-Newman-Keuls procedure (Sokal and Rohlf 1969) were used to test for significant differences between means. However, analysis of cell length and width data was omitted as superfluous because differences in cell size could be demonstrated adequately from area data. In the terminology we have followed Harbach and Knight (1980). Additionally, we have used the terms "outer chorionic cell field" (Linley 1989), and "micropylar dome" (Linley et al. 1991).

Results

Aedes (*Ochlerotatus*) *vigilax* (Figs. 1–3)

Size: as in Table 1. Color: matte black. Overall appearance: asymmetrical in lateral view, ventral side more curved, dorsal side flatter (Fig. 1), widest at about anterior 0.3. In lateral view each outer chorionic cell distinguished by presence, usually, of a single large tubercle, but boundaries of individual cells indistinct. Small tubercles aligned predominantly in circumferential direction (Fig. 1). Micropylar collar not conspicuous.

Chorion, ventral, lateral and dorsal surfaces: all surfaces basically similar, outer chorionic cells irregularly shaped, elongated circumferentially, thus width greater than length (Table 2). Cell dimensions greatest on lateral surface, slightly less on ventral surface, least on dorsal, so cell areas significantly different as indicated (Table 2), but length/width ratio more or less constant.

Cells on ventral surface almost always with single large tubercle, more or less round but sometimes irregularly shaped or compound (Fig. 2a, b). Base of tubercle joined some distance from bottom by bridges from surrounding small tubercles (Fig. 2a, b), cap of tubercle with small, often poorly defined nodules (Fig. 2b, c). Large tubercles in lateral and dorsal cells usually single, but sometimes smaller, or divided (particularly on dorsal surface) into two or three separate, smooth-surfaced tubercles with bridges to neighboring small ones (Fig. 2d, e, f). Mean

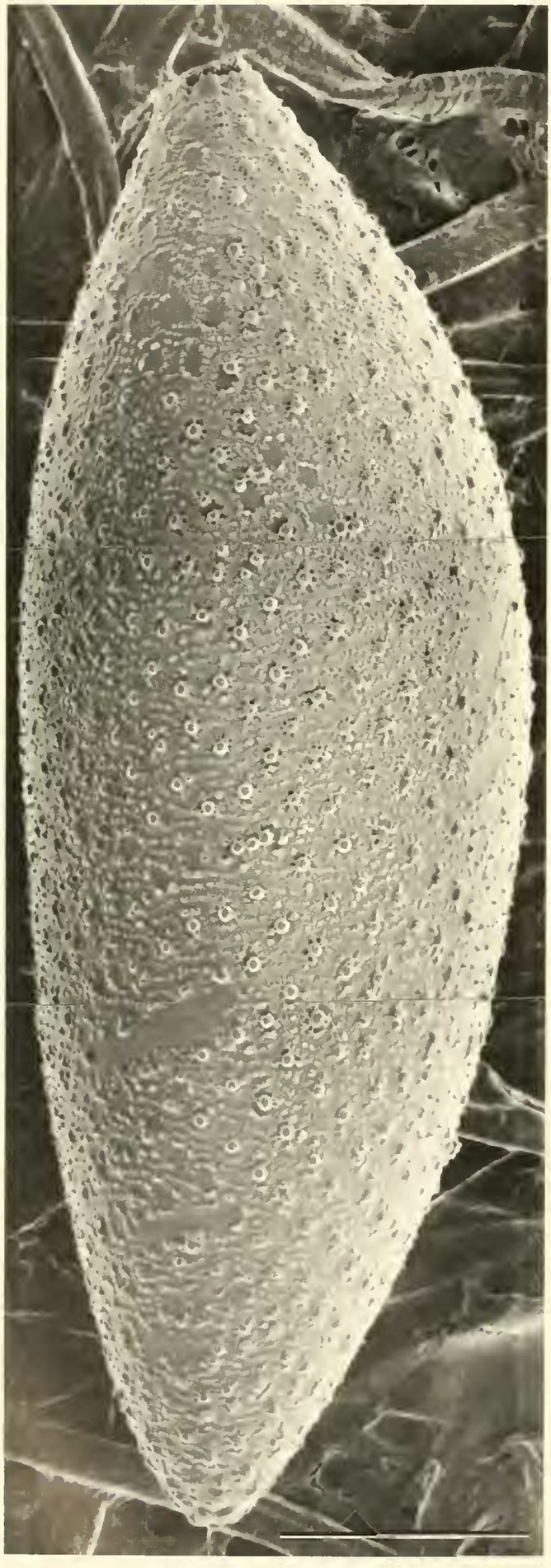

Fig. 1. *Aedes vigilax*. Entire egg, lateral view, ventral side at right, anterior end at top. Scale = 100 μm.

diameter of large tubercles on dorsal surface significantly less than elsewhere (Table 3).

Small tubercles on all surfaces irregular in shape, often difficult to identify individually (therefore not counted or measured), surfaces rough, almost always inclined towards and often forming a bridge to large tubercle (Fig. 2a, b). Small tubercles in cell circumferential extensions usually joined by bridges to one another (Fig. 2a, b). Outer chorionic reticulum on all surfaces usually a fine meshwork, moderately distinct (Fig. 2b, c, f), diameter 3.0–3.3 μm, with central row of small protuberances, diameter 0.2–0.6 μm. Reticulum in some areas on ventral surface sometimes narrower, striations of meshwork less distinct and perforated by small pores (Fig. 2b).

Anterior end, micropyle: chorionic cells smaller towards anterior end, width reduced relative to length (Fig. 3a, b), cell field increasingly obliterated by progressively fused small tubercles, especially just posterior to micropylar collar (Fig. 3b). Cells immediately posterior to collar elongated longitudinally, large tubercles and reticulum less distinct (Fig. 3b). Collar not prominent, anterior edge rounded, continuous or with small gaps (Fig. 3c, d), height 9–12 μm, outer diameter 24–38 μm and highly variable, surface rough (Fig. 3d). Wall width 1.2–9 μm, sometimes very narrow (Fig. 3c), but in some eggs much thicker, with gaps (Fig. 3d). Internal diameter of collar 20–23 μm, inner wall with very shallow excavations (Fig. 3c, d), micropylar disk wide, diameter 13–16 μm, boundary distinct and raised, with more or less round or slightly irregular margin (Fig. 3c, d). Micropylar dome present, not easily distinguished in some eggs, diameter 9.5–12 μm, micropylar orifice unusually small, very slightly trilobed (Fig. 3c), diameter 1.7 μm.

Posterior end: chorionic cells smaller approaching posterior end, widths reduced relative to lengths, small tubercles progressively more fused and united to large tubercles (Fig. 3e, f), reticulum clearly visible

Table 1. Dimensions of eggs of *Ae. vigilax* (n = 7) and *Ae. vittiger* (n = 10).

Species	Length (μm)		Width (μm)		L/W Ratio	
	$\bar{x}$ ± SE	Range	$\bar{x}$ ± SE	Range	$\bar{x}$ ± SE	Range
Ae. vigilax	627.7 ± 4.7	605.7–640.0	201.94 ± 4.2	188.6–217.1	3.11 ± 0.07	2.88–3.36
Ae. vittiger	742.9 ± 3.8	732.3–770.2	352.0 ± 1.1	346.0–356.1	2.11 ± 0.01	2.07–2.15

but its meshwork much less distinct (Fig. 3f).

Aedes (*Ochlerotatus*) *vittiger* (Figs. 4–6)

Size: as in Table 1. Color: satiny black. Overall appearance: shape rhomboidal in ventral (Fig. 4) and dorsal views, asymmetrical in lateral view, ventral surface much more arched, dorsal surface flatter (Fig. 5a), widest just anterior to middle of egg (Fig. 4). All surfaces appear extremely uniform, outer chorionic cells pentagonal or hexagonal, each with a single prominent, round large tubercle (Figs. 4, 5a). Micropylar collar fairly conspicuous (Fig. 4).

Chorion, ventral, lateral and dorsal surfaces: all surfaces very similar (Fig. 5b, c, d), outer chorionic cells somewhat wider than long and L/W ratio decreasing significantly from ventral to dorsal surfaces (Table 2), indicating progressive relative increase in width. Cells in lateral region significantly greatest in area, however, followed by ventral and then dorsal cells (Table 2).

Each cell on all surfaces invariably with a single round, centrally positioned large tubercle (Fig. 5a, b, c), which appears very round at relatively low magnifications (Figs. 4, 5b). Perimeter of tubercle base slightly irregular, vertical walls rough, supporting a more or less round cap ornamented with very clearly defined nodules separated by clear, narrow fissures (Fig. 5e, f, g). Diameter of large tubercles very uniform on each surface, greatest on ventral, least on dorsal, not differing markedly between surfaces, but significant differences present as indicated (Table 3).

Small tubercles quite evenly spaced around margins of cell fields (Fig. 5b, c, d), some cells also with a halo of radially oriented ridges or tiny, nodular tubercles surrounding central large one (Fig. 5d, e, f, g). Numbers of small tubercles (counting only those in outer ring) in each cell as shown (Table 3), significantly different between all three surfaces, but very uniform and not significantly different in diameter (Table 3). Shape of small tubercles variable, tending to be triangular or diamond shaped in cell corners, rectangular along cell margins (Fig. 5d, e, f). Base of each tubercle slightly greater in diameter than cap, walls smooth (Fig. 5e, f, g), cap covered with small nodules,

Table 2. Attributes of outer chorionic cells in eggs of *Ae. vigilax* and *Ae. vittiger* (n = 15). Means followed by same letter do not differ significantly ($P < 0.05$).

Species	Surface	Mean (±SE) Outer Chorionic Cell			
		Length (μm)	Width (μm)	Ratio L/W	Area (μm²)
Ae. vigilax	Ventral	14.6 ± 0.5	33.2 ± 0.7	0.44 ± 0.01a	287.3 ± 9.2a
	Lateral	16.7 ± 0.5	34.7 ± 0.9	0.49 ± 0.02a	300.0 ± 11.6a
	Dorsal	12.7 ± 0.3	28.3 ± 0.8	0.45 ± 0.01a	210.9 ± 8.2b
Ae. vittiger	Ventral	25.4 ± 0.6	26.3 ± 0.8	0.98 ± 0.04a	436.3 ± 14.3a
	Lateral	24.6 ± 0.8	34.6 ± 0.7	0.72 ± 0.03b	537.9 ± 21.1b
	Dorsal	20.8 ± 0.6	30.7 ± 0.6	0.68 ± 0.02c	386.4 ± 9.8c

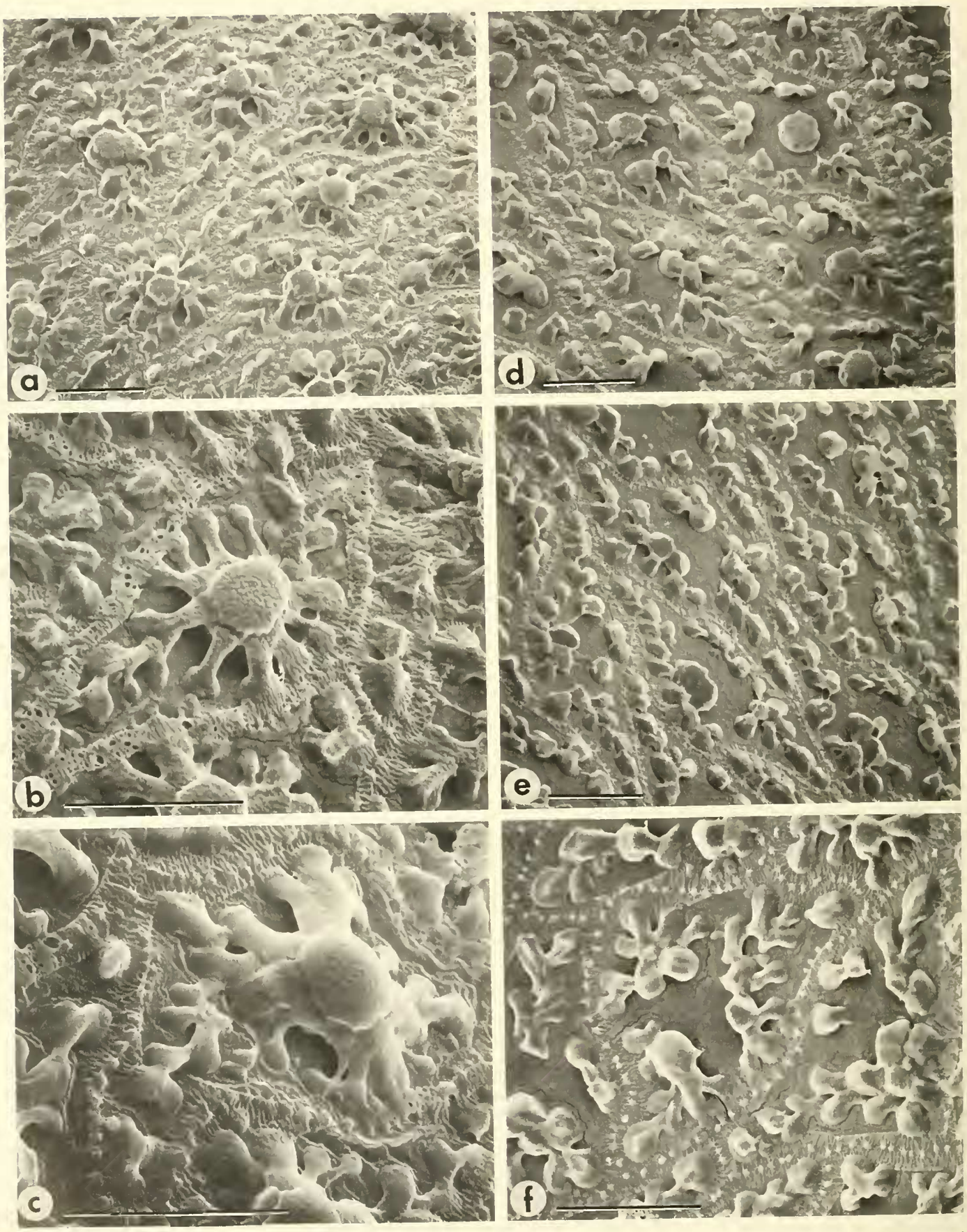

Fig. 2. *Aedes vigilax*. (a) Chorionic cells, ventral surface, middle of egg; (b) chorionic cell detail, ventral surface; (c) detail of chorionic reticulum, ventral surface; (d) chorionic cells, lateral surface, middle of egg; (e) chorionic cells, dorsal surface, middle of egg; (f) chorionic cell detail, dorsal surface. Scale = 10 μm.

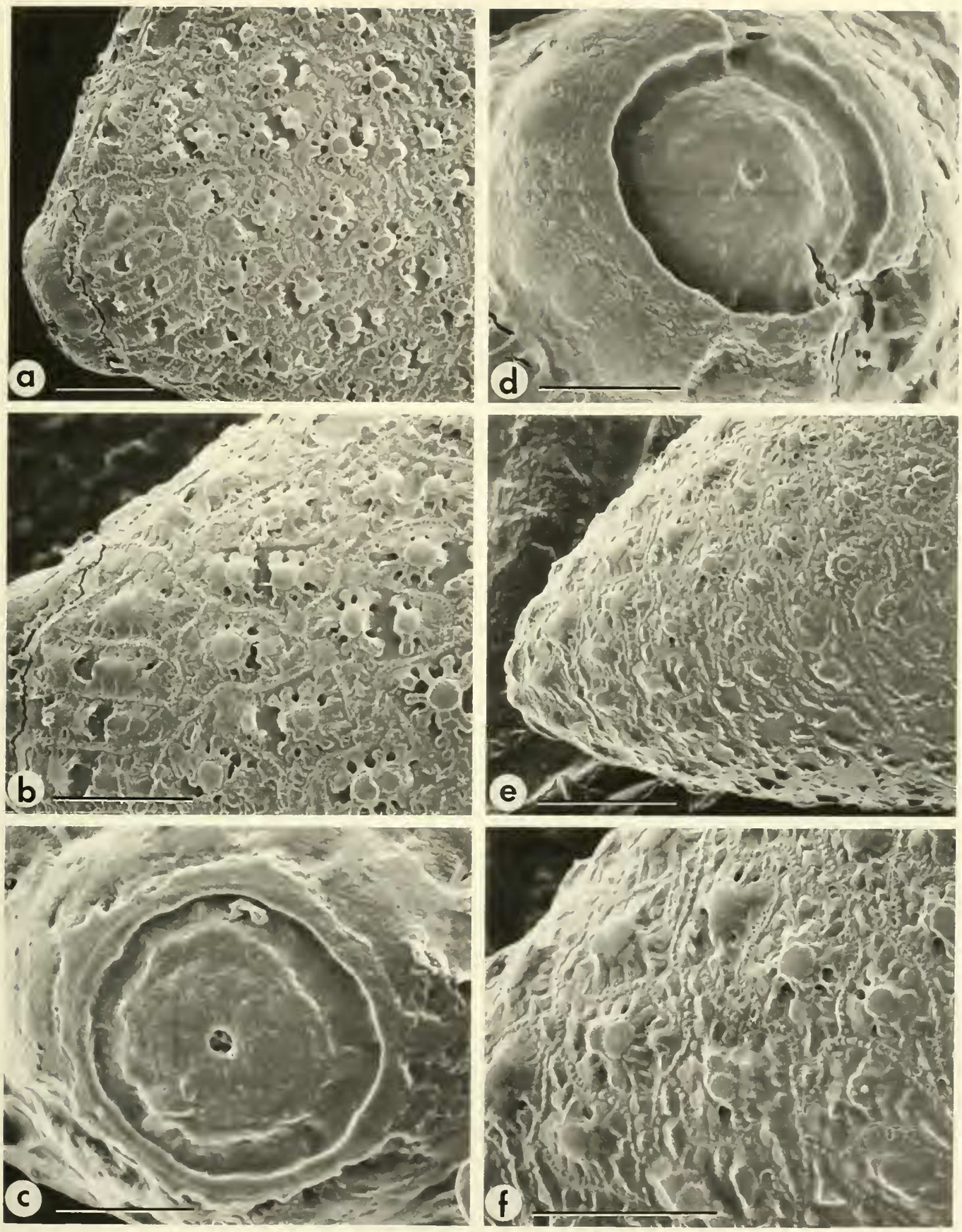

Fig. 3. *Aedes vigilax*. (a) Anterior end, lateral view; (b) anterior end, chorionic cell detail; (c) micropylar apparatus, showing continuous collar; (d) micropylar apparatus with discontinuous collar and showing micropylar dome; (e) posterior end, lateral view; (f) posterior end, chorionic cell detail. Scale = 20 μm (a, b, e, f), = 10 μm (c, d).

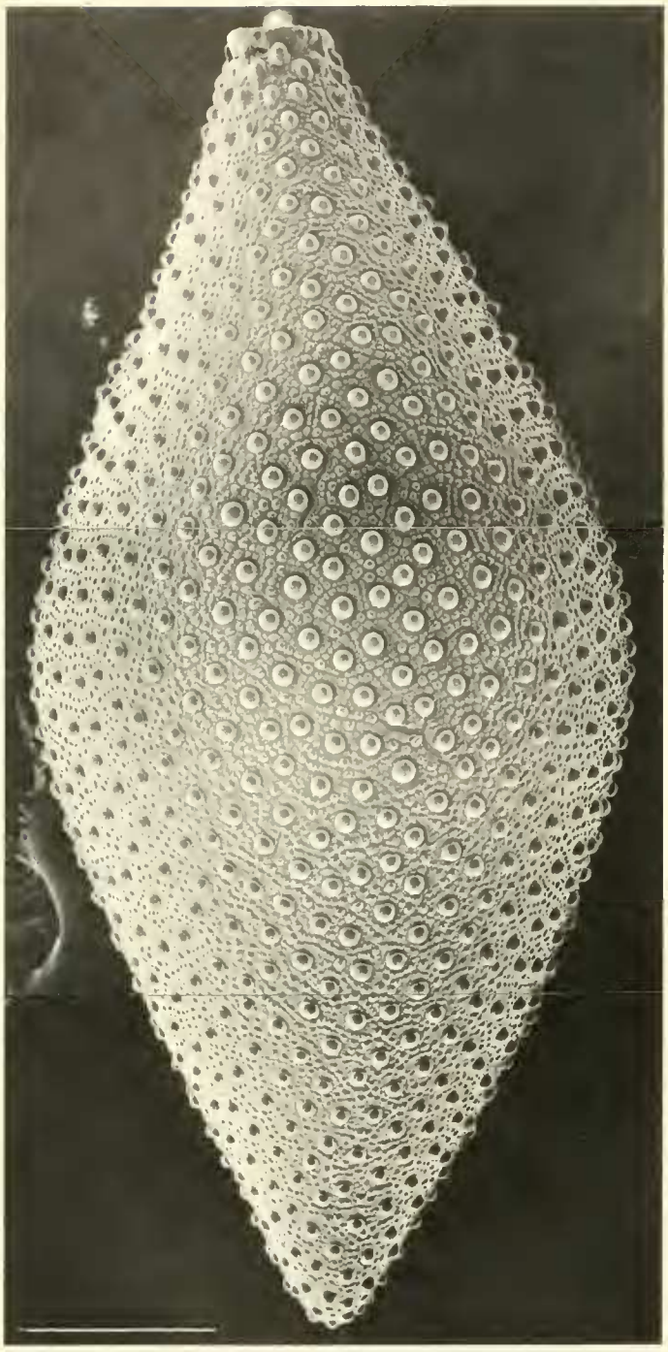

Fig. 4. *Aedes vittiger*. Entire egg, ventral view, anterior end at top. Scale = 100 μm.

which are smaller and more round than those on large tubercles (Fig. 5g), dividing fissures well defined and uniform in width. Cell fields partly smooth, especially bordering peripheral small tubercles, but much of area covered with a more or less continuous fine reticulation (Fig. 5g), similar to that in outer chorionic reticulum. Reticulum structured as just indicated, diameter 2–3.2 μm, surface usually with very shallow indentations (Fig. 5e, f), and a central line of tiny papillae, diameter 0.2–0.6 μm.

Anterior end, micropyle: chorionic cells diminish in size immediately posterior to micropylar collar, cell fields generally smoother and central papillae in reticulum less distinct or absent (Fig. 6a, b). Large tubercles immediately posterior to collar somewhat longitudinally elongated, becoming continuous with collar (Fig. 6a, b). Collar fairly prominent, lateral and anterior faces lumpy (Fig. 6a, d, e), surface slightly rough (Fig. 6e). Collar often a complete ring (Fig. 6d, e), but occasionally with one to three gaps (Fig. 6c), height 8–11.5 μm, outer diameter 51–55 μm, wall width fairly uniform (gaps excepted), 7–13 μm. Collar internal diameter 32–37 μm, inner wall with shallow excavations, walls with vertical striations (Fig. 6d, e), micropylar disk fairly distinct, slightly raised, outline irregular, surface rough (Fig. 6d, e), diameter 16–19 μm. Micropylar dome also visible, diameter 11–12 μm, orifice distinctly tri-lobed (Fig. 6e), diameter 2.1 μm.

Table 3. Attributes of the large (n = 15) and small (n = 30) outer chorionic tubercles in eggs of *Ae. vigilax* and *Ae. vittiger*. Means followed by same letter do not differ significantly ($P < 0.05$).

Species	Surface	Mean (±SE)		
		Large Tubercles Diameter (μm)	Small Tubercles	
			No.	Diameter (μm)
Ae. vigilax	Ventral	4.7 ± 0.1a		
	Lateral	4.4 ± 0.1a	not determined	
	Dorsal	2.4 ± 0.1b		
Ae. vittiger	Ventral	9.6 ± 0.1a	13.5 ± 0.3a	2.7 ± 0.2a
	Lateral	9.4 ± 0.2ab	15.1 ± 0.5b	2.6 ± 0.2a
	Dorsal	9.0 ± 0.1b	10.7 ± 0.3c	2.9 ± 0.2a

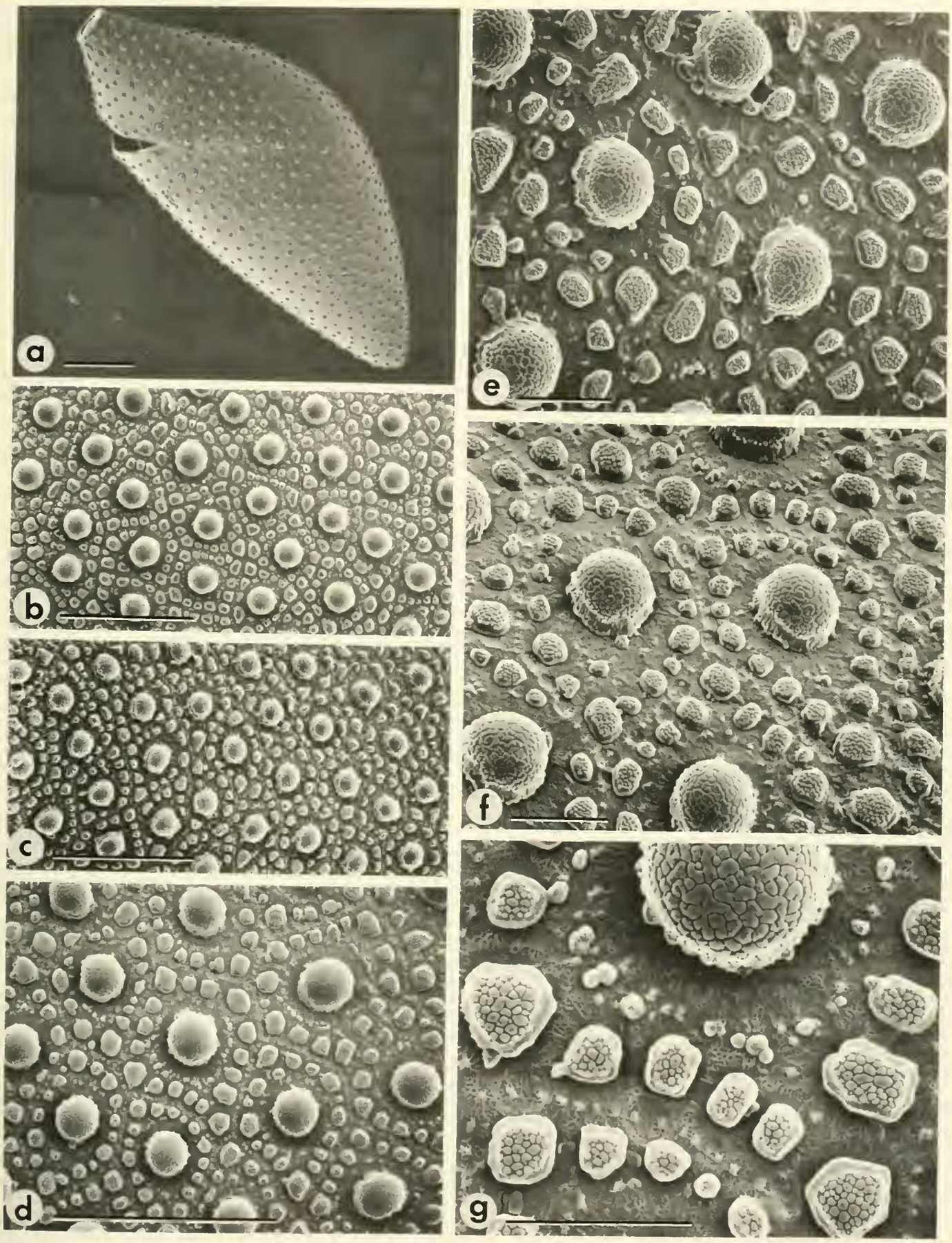

Fig. 5. *Aedes vittiger*. (a) Entire egg, lateral view, anterior end at left; (b) chorionic cells, ventral surface, middle of egg; (c) chorionic cells, dorsal surface, middle of egg; (d) chorionic cells, lateral surface, middle of egg; (e) chorionic cell detail, dorsal surface; (f) chorionic cell detail, lateral surface; (g) detail of tubercles and chorionic reticulum, ventral surface. Scale = 100 μm (a), = 50 μm (b, c, d), = 10 μm (e, f, g).

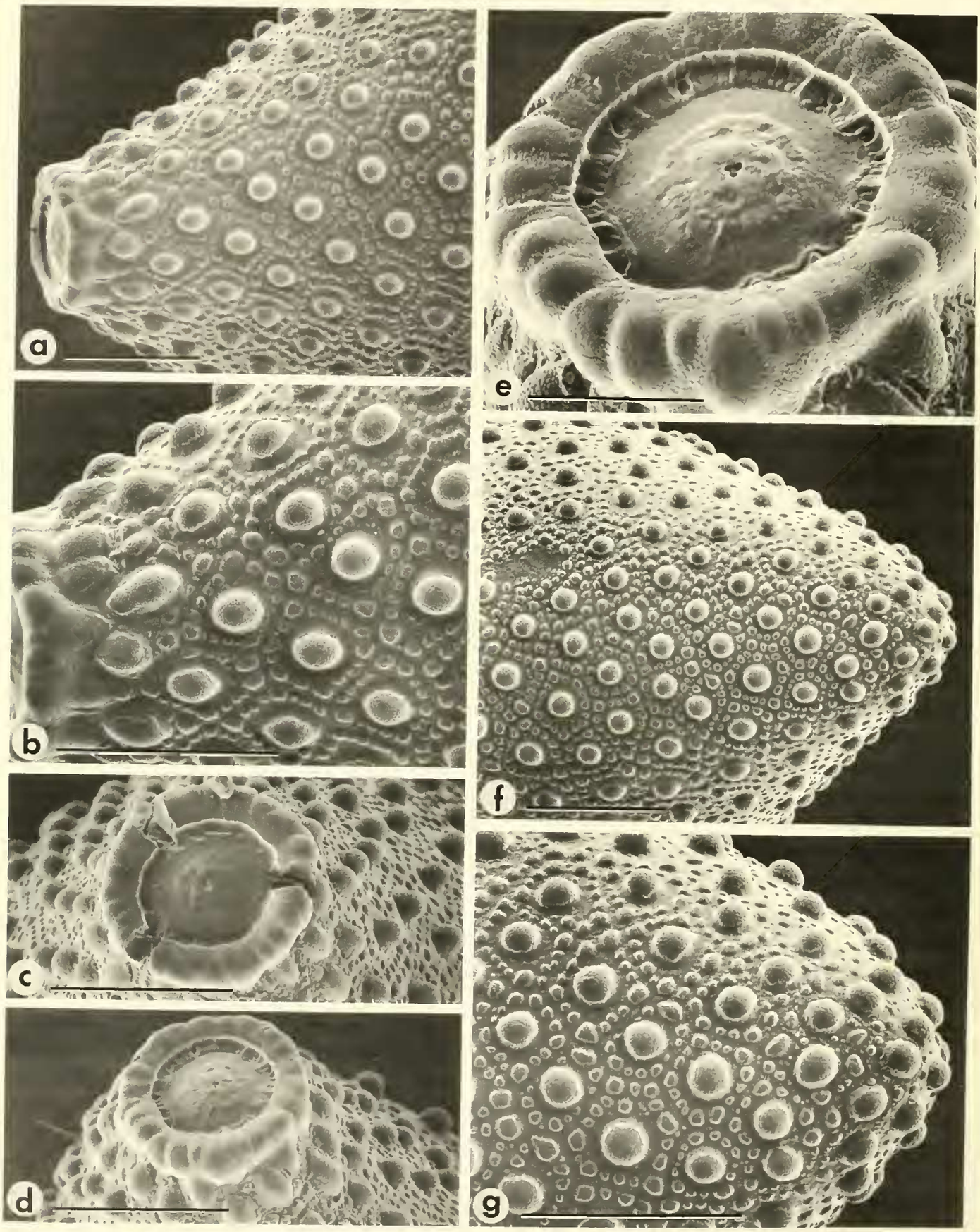

Fig. 6. *Aedes vittiger*. (a) Anterior end, lateral view; (b) anterior end, chorionic cell detail; (c) anterior end and micropylar apparatus with discontinuous collar; (d) anterior end and micropylar apparatus, continuous collar; (e) detail of micropylar apparatus, showing micropylar disk and dome; (f) posterior end, lateral view; (g) posterior end, chorionic cell detail. Scale = 50 μm (a, b, c, d, f, g), = 20 μm (e).

Posterior end: chorionic cells progressively smaller towards posterior end, numbers of small tubercles fewer, cell fields smoother, reticulum less distinct with central papillae not easily visible (Fig. 6f, g). Identity of individual chorionic cells with single large tubercle distinct even at very end of egg (Fig. 6g).

Discussion

The relative uniformity of structure over all surfaces of *Ae. vigilax* eggs is in keeping with observations suggesting that females of this species do not cement their eggs to the oviposition surface. Hamlyn-Harris (1933) reported that *Ae. vigilax* deposits eggs both on salt water and on damp surfaces subject to flooding, while Sinclair (1976) described the preferred site as damp soil with low covering vegetation, but not bare mud. Kay and Jorgensen (1986) recovered eggs from mangrove pneumatophores and from the bases of marine couch plants, which might suggest attachment of the eggs, but they also remarked that eggs were easily dislodged by agitation or a fine jet of water. Freedom from attachment may be important for these eggs as they may be carried some distance on incoming tidal flow and left in isolated pools as waters recede (Hamlyn-Harris 1933). The exact oviposition sites preferred by female *Ae. vittiger* are unknown, but certainly the extreme surface uniformity of its eggs and absence of cement from eggs laid in the laboratory indicate no attachment to the substrate.

A seemingly unusual feature of *Ae. vigilax* eggs is the rather complex shape of the outer chorionic cells, in which there are tongue-like circumferential extensions on each side of the cell. Olson and Meola (1976) described such cells on the egg of *Ae.* (*Och.*) *sollicitans* (Walker) and recent observations of eggs of several other species indicate that this may be a fairly common configuration within the subgenus *Ochlerotatus.* In eggs of *Ae.* (*Och.*) *procax* (Skuse), for example, the tongue-shaped extensions are highly developed (J. R. Linley, M. J. Geary and R. C. Russell, unpublished observations). Cells similarly shaped are present over the entire surface of *Ae.* (*Och.*) *scapularis* (Rondani) eggs (J. R. Linley and F. J. Burton, unpublished) and on the lateral and dorsal surfaces in *Ae.* (*Och.*) *infirmatus* Dyar and Knab (Linley 1990). There is apparently some advantage associated with this shape, either during egg development in the ovary, where the follicular epithelial cells must also be so formed, or after the egg is laid. Under the stereomicroscope, the complex outline of the cells can be distinguished by reflected light at high ($\geq 80\times$) magnification and this might be quite useful for rapid and easy identification without resort to electron microscopy or hatching to obtain larvae. According to Hamlyn-Harris (1933), *Ae.* (*Mucidus*) *alternans* (Westwood) may share breeding habits with *Ae. vigilax,* but the substantially more rhomboidal shape of its egg and distinctly different chorionic cell structure (Linley et al. 1991) render it easily distinguishable stereomicroscopically from *Ae. vigilax.*

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Literature Cited

Dobrotworsky, N. V. 1965. The Mosquitoes of Victoria (Diptera: Culicidae). Melbourne University Press, Victoria.

Fenner, F. and F. N. Ratcliffe. 1965. Myxomatosis. Cambridge University Press, London, New York and Ibadan.

Hamlyn-Harris, R. 1933. Some ecological factors involved in the dispersal of mosquitoes in Queensland. Bulletin of Entomological Research 24: 229–232.

Harbach, R. E. and K. L. Knight. 1980. Taxonomists' Glossary of Mosquito Anatomy. Plexus Publishing, Marlton, New Jersey.

Kay, B. H. and W. K. Jorgensen. 1986. Eggs of *Aedes*

vigilax (Skuse) and their distribution on plants and soil in south east Queensland saltmarsh. Journal of the Australian Entomological Society 25: 267–272.

Lee, D. J., M. M. Hicks, M. Griffiths, R. C. Russell, and E. N. Marks. 1984. The Culicidae of the Australasian Region. Volume 3. Entomology monograph No. 2, Commonwealth Department of Health, Australian Government Publishing Service.

Linley, J. R. 1989. Comparative fine structure of the eggs of *Ae. albopictus, Ae. aegypti* and *Ae. bahamensis* (Diptera: Culicidae). Journal of Medical Entomology 26: 510–521.

———. 1990. Scanning electron microscopy of the eggs of *Aedes vexans* and *Aedes infirmatus* (Diptera: Culicidae). Proceedings of the Entomological Society of Washington 92: 685–693.

Linley, J. R., M. J. Geary, and R. C. Russell. 1991. The eggs of *Aedes* (*Verrallina*) *funereus, Aedes* (*Finlaya*) *notoscriptus* and *Aedes* (*Mucidus*) *alternans* (Diptera: Culicidae). Proceedings of the Entomological Society of Washington 93: 592–612.

Marks, E. N. 1967. An atlas of common Queensland mosquitoes, *with* a guide to Queensland biting midges by E. J. Reye. University of Queensland Bookshop, St. Lucia, Brisbane, Australia.

McLean, D. M. 1953. Transmission of Murray Valley encephalitis virus by mosquitoes. Australian Journal of Experimental Biology and Medical Science 31: 481–490.

Olson, J. K. and S. M. Meola. 1976. Variations in chorionic sculpturing of eggs of *Aedes sollicitans* (Walker). Annals of the Entomological Society of America 69: 96–100.

Pillai, J. S. 1962. Factors influencing egg survival in *Aedes* eggs with special reference to some Victorian species (Diptera: Culicidae). Ph.D. thesis, University of Melbourne, Australia, 1962.

Reynolds, J. L. 1961. The physiology and ecology of the immature stages of the salt marsh mosquito *Aedes vigilax* Skuse (Diptera: Culicidae). M.Sc. thesis, University of New South Wales, Australia, 1961.

Sinclair, P. 1976. Notes on the biology of the salt-marsh mosquito, *Aedes vigilax* (Skuse) in south-east Queensland. Queensland Naturalist 21: 134–139.

Sokal, R. R. and F. J. Rohlf. 1969. Biometry. W. H. Freeman and Company, New York.